

Anisotropic diffusion of spherical particles in closely confining microchannels

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Abstract

We present here the measurement of the diffusivity of spherical particles closely confined by narrow microchannels. Our experiments yield a 2D map of the position-dependent diffusion coefficients parallel and perpendicular to the channel axis with a resolution down to 129 nm . The diffusivity was measured simultaneously in the channel interior, the bulk reservoirs as well as the channel entrance region. In the channel interior we found strongly anisotropic diffusion. While the perpendicular diffusion coefficient close to the confining walls decreased down to approximately 25% of the value on the channel axis, the parallel diffusion coefficient remained constant throughout the entire channel width. In addition to the experiment, we performed finite element simulations for the diffusivity in the channel interior and found good agreement with the measurements. Our results reveal the distinctive influence of strong confinement on Brownian motion which is of significance to microfluidics as well as quantitative models of facilitated membrane transport.

INTRODUCTION

Diffusion in close confinement is paramount to transport across biological membranes and understanding the physical processes governing transport is of great relevance for designing drugs [1]. Many molecules are transported across the membrane by passive diffusion through proteins that form long and narrow channels. Channel-facilitated diffusion has been studied experimentally [2–4] as well as theoretically [5–14] and interpreting the models requires knowledge of the spatial dependence of diffusion coefficients inside the channel and at the entrance regions, either explicitly in the continuous models or implicitly in the form of diffusive hopping constants for discrete models. Besides the relevance to biological transport, it is also of interest in the study of physical phenomena such as entropic particle transport in corrugated channels for particle separation [15, 16]. In the confinement of bounding walls, the diffusion coefficients of particles are decreased by viscous interactions with the walls as compared to the value in an infinite fluid. This hindered diffusion has been studied extensively for planar geometries involving spherical particles moving either above a single or in between two plane walls [17–29]. However, to our knowledge, only one experimental study investigates position-dependent hindered diffusion in the presence of curved boundaries [30]. The authors studied the hindered diffusion of spherical particles inside closed cylinders that were considerably larger than the particles. Experiments on the diffusion of particles in closely-confining channels [31] have been limited to effectively infinitely long channels and diffusion along the channel axis. So far, measurements of the position-dependent diffusion coefficients in closely-confining, finite length channels are lacking completely.

METHODS

In this Letter we report the measurement of a complete 2D map with 129 nm resolution of the position-dependent diffusion coefficients of spherical particles. The polystyrene spheres (Polysciences (Warrington, PA), (505 ± 8) nm diameter) moved through an array of parallel, closely-confining microchannels of semi-elliptical cross-section (approximately $5 \mu\text{m}$ length and $1 \mu\text{m}$ width and height) separating two bulk reservoirs (Figure 1(a)). Our data covers the channel interior as well as the entrance regions and the bulk reservoirs.

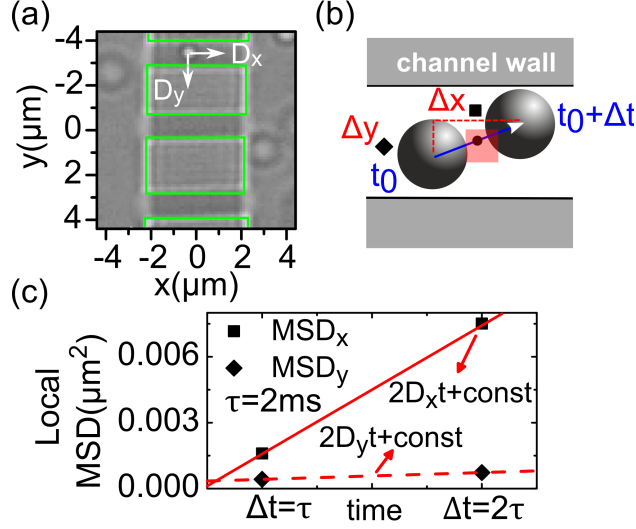


FIG. 1. Method for measuring local diffusion coefficients. (a) Particles diffusing in the microfluidic chip, containing two bulk reservoirs connected by three parallel microfluidic channels, are imaged by bright-field video microscopy. The channel edges are marked by the lines. We choose the coordinates such that the diffusion coefficients parallel and perpendicular to the channel walls are D_x and D_y respectively. (b) Displacements of tracked particles after time lags of one and two video frames are assigned to the bin of the midpoint of the displacement vector (marked by the box). (c) This yields the first two points of the MSD -vs- t -curves in both x - and y -direction for each bin. The slope of the linear fit yields the diffusion coefficients in x and y respectively.

The channels were realized in a microfluidic chip made in Polydimethylsiloxane (PDMS) via replica molding [4, 32]. Briefly, for creating the mold an array of platinum wires was deposited on a silicon substrate via focused ion beam. The wire cross section was measured *in situ* by slicing the wire at one end, tilting the sample at 63° and imaging with a scanning electron microscope (SEM). Conventional photolithography, replica molding and PDMS bonding to a glass slide were carried out to define $16 \mu m$ thick reservoirs separated by a PDMS barrier and connected by the array of channels obtained as a negative replica of the platinum wires. The chip was filled with the particles dispersed in a 5 mM KCl solution and continuously imaged through an oil immersion objective ($100\times$, 1.4 N.A., UPLSAPO, Olympus). Illumination was provided from above by an LED light (Thorlabs MWLED). The transmitted light was collected by the objective and coupled to a CMOS camera (frame rate of 500 fps and magnification of $\sim 8 px/\mu m$). With the objective having a depth of

focus of approximately $2\ \mu\text{m}$ and the focal plane close to the glass cover slide, particles were always tracked in proximity to at least one bounding wall. Experiments were automated using a custom-made LabVIEW program for positioning and video acquisition [4]. The temperature inside the chip during the experiment was monitored using a digital thermometer (RS Components, K-type thermocouple, 0.2% accuracy). Particle trajectories in 2D were extracted from the microscopy videos via a custom-written automated tracking algorithm with accuracies better than 20 nm inside the channels. Local diffusion coefficients were determined from a linear fit to local *MSD*-vs-time curves [33] (Figure 1(b),(c)). In short, we followed particles and measured their displacements for time lags of one and two frames respectively. These displacements were assigned to the position bin of the mid-point of the displacement vector. Subsequently, in each bin the measured values were averaged to give the first two points of local *MSD*-vs-time curves for both *x*- and *y*-direction (Figure 1(c)). The slope of the linear curve connecting these two points yields the diffusion coefficients. For creating the 2D map we binned the *xy*-positions into single camera pixel bins ($129 \times 129\ \text{nm}^2$). Trajectories of particles exploring the channels and the bulk were recorded for 80 min of video corresponding to 2.453 million frames and 5.506 million tracked particle positions.

For our numerical simulations we used COMSOL Multiphysics 4.3b with the creeping flow module to solve Stokes' equation by the Finite Element Method with adaptive mesh size. The system treated was a spherical particle moving in an infinitely long channel of semi-elliptical cross-section. The particle was positioned on different grid points in the cross-sectional *yz*-plane and the viscous friction tensor $\boldsymbol{\nu}$ calculated for each position. We imposed no-slip boundary conditions on the sphere surface and the channel walls. Furthermore, we utilised a common computational approach[34] and switch to the frame of reference of the particle. Thereby the walls become moving which is mathematically treated as a slip-velocity on the wall surface ($\vec{v} = v\vec{e}_x$ or $\vec{v} = v\vec{e}_y$, corresponding to parallel and perpendicular diffusion). To compare the simulations with the measured diffusion coefficients in the channel interior we used the Stokes-Einstein relation [35] which gave the ratios $D_x(y, z)/D_0$ and $D_y(y, z)/D_0$ (see Supplementary Information for details). For calculating the bulk diffusivity, D_0 , we inserted the temperature measured inside the chip during the experiment, $T = 301.7\ \text{K}$, the particle radius $a = 250\ \text{nm}$ and viscosity of water $\eta(T)$ [36] into the Stokes-Einstein equation, giving $D_0 = 1.08\ \mu\text{m}^2/\text{s}$. For arriving at the perpendicular dependence of diffusivity,

$D(y)$, we averaged the values $D(y, z)$ over the entire z -range by random sampling in order to avoid mesh artifacts. In the experiments, our measured diffusion coefficients represent as well values averaged over the entire z -range of 700 nm accessible to the particles. It is important to note that the strong confinement in z -direction combined with the semi-elliptical cross-section leads to suppressed axial position fluctuations inside the channels.

The PDMS channel width was determined optically from the microscopy videos as well as from considering the width over which particles were tracked inside the channel. The widths of both measurement methods agreed and we found values of $(1.15 \pm 0.13) \mu m$ for the bottom two channels and $(1.02 \pm 0.13) \mu m$ for the top channel, thus all three channels had the same width within measurement accuracy. For the numerical simulations we used a width of $1.2 \mu m$ and assumed that the semi-elliptical cross-section of the platinum wires was preserved.

RESULTS

A 2D map of diffusivity parallel to the channel axis (D_x) is shown in Figure 2(a) (for the 2D map of D_y see the Supplementary Information, Figure S.1). We found a significantly reduced diffusivity inside the channels as compared to the values measured in the bulk. To quantify this further, we measured the dependence of diffusion coefficients along the channel axis, $D_{x,y}(x)$. For this we averaged over the three bins closest to the channel axis for each channel and x -position (for the top channel the total number of bins in y -direction was even so we averaged over the two closest bins). The data for the central channel is shown in Figure 2(b) and (c) (data for the other two channels are shown in the Supplementary Information, Figure S.2). The diffusivity dependence was qualitatively the same for both D_x and D_y in all three channels. The diffusion coefficients showed an approximately constant value in the bulk followed by an extended transition region in which it decreased towards a plateau of lower diffusivity inside the channel. We evaluated $D_{x,y}(x)$ only for $x \in [-4 \mu m, 4 \mu m]$ to avoid edge effects of the finite tracking region (see [33] for more details). Within measurement accuracy, the average diffusivity inside the channels, $D_{x,ch}, D_{y,ch}$, was the same for all three channels studied. The detailed values can be found in Table I. The length of the transition region between bulk and channel was around $1 - 1.5 \mu m$, without significant

differences between channels within measurement accuracy. For perpendicular diffusivity, however, the plateau inside the channel was slightly shorter ($\approx 0.5 \mu m$, i.e. one particle diameter) than that of the parallel diffusivity D_x . Indeed, in order to reduce D_y , the particle has to be fully enveloped by the channel. This explains the small difference in transition length scales for D_x and D_y . Furthermore, $D_{y,ch}$ was lower than $D_{x,ch}$ due to the motion perpendicular to the channel walls being more strongly confined than that in the parallel direction. We noticed that the diffusivity in both x - and y -direction in our bulk reservoirs reached a value of $D_{xy} = (0.74 \pm 0.06) \mu m^2/s$ rather than the Einstein-Stokes value of $D_0 = 1.08 \mu m^2/s$. This can be attributed to the hydrodynamic friction exerted by the glass slide. Using Goldman's theory [20] we estimated the average hydrodynamic separation z between the particle centers and the glass surface [26]. Inverting the theoretical relationship for the diffusivity parallel to a plane wall, $D_{xy}(a/z)/D_0$ (series expansion from Happel [34]), yielded an average distance of $z = (370 \pm 30) nm$.

For the channel interior, we calculated the dependence of the diffusivity on the distance b from the channel axis, $D_x(b)$ and $D_y(b)$. We averaged over all bins between $x = -2 \mu m$ and $x = +2 \mu m$ for each y -value up to the channel walls. The perpendicular diffusivity D_y showed an approximately parabolic decrease from the axis towards the channel walls. By fitting a parabola to the data we determined the position of the channel axis ($b = 0$) from the maximum of the parabola at sub-pixel resolution as well as the on-axis diffusivity $D_y(b = 0)$. We determined the parallel on-axis diffusivity $D_x(b = 0)$ as the measured $D_x(b)$ value closest to the center for an uneven number of bins in y -direction or the average of the two closest bins in the case of an even number of bins. The normalized dependences $D_y(b)/D_y(0)$ and $D_x(b)/D_x(0)$ for the central channel are shown in Figure 3 (data for the other two channels are shown in the Supplementary Information, Figure S.3).

The data for D_y shows that the diffusion coefficient is at a maximum in the channel center. As the particle is moving closer to the channel wall, D_y drops significantly, as expected when the particle approaches the channels walls (see Supplementary Information and Figure S.3 for a further discussion).

In stark contrast, the diffusivity parallel to the channel axis (D_x) remained almost constant throughout the entire channel width (circles in Figure 3). This is opposed to expectations based on hindered diffusion in proximity to plane walls. To our knowledge, the surprising behavior we found here has not previously been observed experimentally in microfluidic

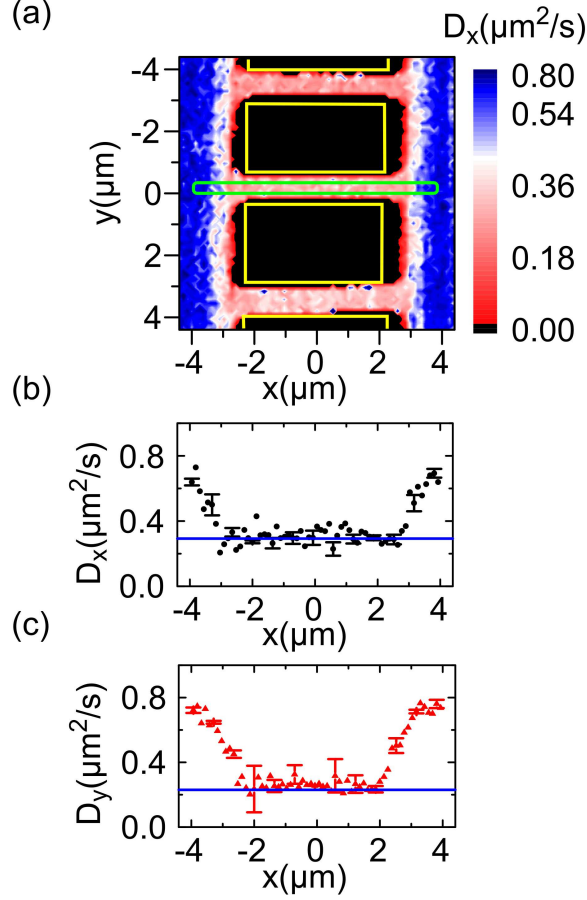


FIG. 2. Local diffusion coefficients and their dependence along the channel axis. (a) The position-dependent diffusion coefficients parallel to the channel axis (D_x) are presented in a color map. The channel edges are marked by the lines. The channels appear longer and thinner in the color map because it is based on the center position of finite size spheres. The color map for the perpendicular diffusivity (D_y) can be found in the Supplementary Information, Figure S.1. (b) and (c) The diffusivity dependence along the channel axis ($D_{x,y}(x)$) is calculated in the marked box by averaging over the three bins for each x -value. Error bars are the standard deviation in between bins. For clarity error bars are only shown for points spaced 650 nm. The parallel diffusivity $D_x(x)$ is shown in (b) and the perpendicular one $D_y(x)$ in (c).

channels. Only analytical and numerical studies on the hydrodynamic drag force experienced by spherical particles translating in closely-fitting cylindrical channels [37, 38] have predicted this kind of dependence. Thus, our experiments allowed for the first qualitative experimental testing of their predictions in very close confinements on the submicron scale. Despite the lack of an analytical solution due to the cross-sections of our channels being semi-elliptical

rather than cylindrical we could compare our measurements to our finite element simulations and found good agreement (absolute values for D_x and D_y are shown in Table I, the b -dependence in Figure 3). This shows that hindered diffusion behaves qualitatively differently in closely confining channels as compared to more extended geometries due to hydrodynamic interactions determined by the microchannel geometry.

D_x	experimental $D_{x,ch} (\mu m^2/s)$	simulation $D_{x,ch} (\mu m^2/s)$	D_y	experimental $D_{y,ch} (\mu m^2/s)$	simulation $D_{y,ch} (\mu m^2/s)$
top channel	0.27 ± 0.05	0.293 ± 0.002	top channel	0.24 ± 0.05	0.23 ± 0.04
central channel	0.32 ± 0.04	0.293 ± 0.002	central channel	0.26 ± 0.03	0.23 ± 0.04
bottom channel	0.31 ± 0.03	0.293 ± 0.002	bottom channel	0.29 ± 0.03	0.23 ± 0.04

TABLE I. Average parallel and perpendicular diffusion coefficients (D_x and D_y) for all channel interiors. Experimental values were averaged from the $D_{x,y}(x)$ curves in the plateau region between $x = -2 \mu m$ and $x = +2 \mu m$. Given are the average values and the standard deviation between the different points along the x -axis. The simulation values were averaged from the cross-sectional values $D_{x,y}(y, z)$ over the same y - and z -range as the experimental ones. The simulation errors given are the standard error of the mean over the different sampling points.

CONCLUSIONS

In summary, we presented the detailed measurement of the position-dependent diffusion coefficients of spherical particles closely confined by finite length channels in directions parallel and perpendicular to the channel axis. Of particular interest to models of channel-facilitated diffusion is the determination of the dependence along the channel axis, $D_x(x)$, showing that diffusion in the channel interior behaves as if the channels were infinitely long with an almost constant diffusivity throughout the entire channel length.

Furthermore, we observed the parallel diffusivity to remain approximately constant throughout the entire channel width in contrast to the perpendicular diffusivity that decreased towards the channel walls.

We expect that our findings will stimulate further studies of the special features of Brown-

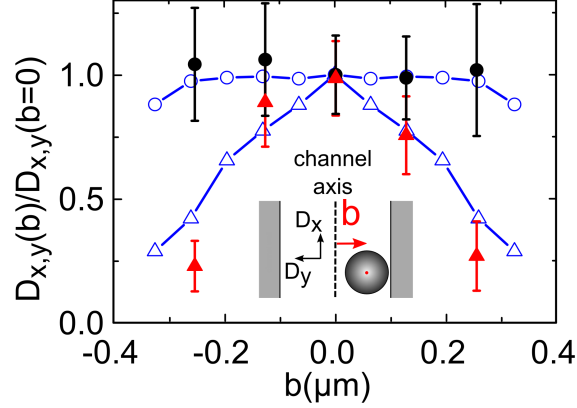


FIG. 3. Diffusion coefficient dependence along the channel width for the central channel. The filled circles \bullet are the measured diffusion coefficients parallel to the channel axis (D_x) and the filled triangles \blacktriangle the ones in perpendicular direction (D_y). The error bars are the standard deviations between the different bins that were averaged over. The lines with empty symbols are values from the numerical simulations for D_x (\circ) and D_y (\triangle). The inset illustrates the definition of the coordinate system.

ian motion arising in strong confinement which is common place in cellular environments. Besides this potential for exciting new insights into biophysics, the physical process of confined Brownian motion is strongly linked to low Reynolds number hydrodynamics in closely confining environments as governing flow in the thriving fields of micro- and nanofluidics. Our results could be of interest to efficiently control particle transport in technological applications as e.g. the construction of drift ratchets for particle sorting.

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